UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY
AND POLLUTION PREVENTION

OFFICE OF PESTICIDE PROGRAMS
REGISTRATION DIVISION (7505P)

July 27, 2016

MEMORANDUM:

Subject:	Name of Pesticide Product:	T2.200 FOR DOGS
	EPA Reg. No. /File Symbol:	91384-G
	DP Barcode:	DP 432710
	Decision No.:	511951
	Action Code:	R315
	Submission:	#983221
	PC Codes:	109701 (Permethrin: 44.00%)
		129099 (Imidacloprid: 8.80%)
		129032 (Pyriproxyfen: 0.44%)

From: Byron T. Backus, Ph.D., Toxicologist
CITAB
Registration Division (7505P)

Byron T. Backus
July 27, 2016

Through: Masih Hashim, Ph.D., Team Leader, Toxicology
CITAB
Registration Division (7505P)

JCR for

To: Elizabeth Fertich, RM 04
IVB1
Registration Division (7505P)

Registrant: CAP IM SUPPLY, INC.

FORMULATION FROM LABEL:

Active Ingredient(s):	by wt.
129099 Imidacloprid	8.80%
109701 Permethrin	44.00%
129032 Pyriproxyfen	0.44%
Other Ingredients:	46.76%
TOTAL	100.00%

ACTION REQUESTED: "The registrant has submitted additional companion animal data in response to the 10-day letter sent on 3/8/16. The deficiency letter was sent to notify the registrant of the issues found in the review dated 2/19/16 (DP 431032). Please review the data and determine if it addresses the deficiencies identified in DP 431032… The following items are attached: 1) cover letter; 2) initial review – DP 431032; 3) hard copies of new studies."

BACKGROUND:

The material received includes a cover letter (MRID 49866900) and two MRIDs: 49866901 (Erasmus, H. (2016) A Target Animal Safety Study of T2 Applied Topically to Adult Dogs: Final Report. Project Number: CV/15/154, PN1767. Unpublished study prepared by ClinVet International (Pty) Limited. 253p.) and 49866902 (Erasmus, H. (2016) A Target Animal Safety Study of T2 Applied Topically to Puppies: Final Report. Project Number: PN1767, CV/15/155. Unpublished study prepared by ClinVet International (Pty) Limited. 152p.). MRIDs 49866901 and 49866902 are responses to a request for additional information and clarifications in a previous CITAB review (dated February 19, 2016) of two studies (MRID 49788721: Erasmus, H. (2015) A Target Animal Safety Study of T2 Applied Topically to Adult Dogs Final Report. Project Number: CV/15/154, PN1767. Unpublished study prepared by ClinVet International (Pty) Ltd. 1963p. and MRID 49788722: Erasmus, H. (2015) A Target Animal Safety Study of T2 Applied Topically to Puppies Final Report. Project Number: CV/15/155, PN1767. Unpublished study prepared by ClinVet International (Pty) Ltd. 1478p).

COMMENTS AND RECOMMENDATIONS:

1. The 44-day companion animal safety study with adult beagles (MRIDs 49788721, 49866901) has been classified as acceptable. This study supports the proposed use on adult dogs, although the proposed minimum weight (based on the results of the puppy study in MRIDs 49788722 and 49866902) should be raised to 5 lbs, and the minimum weight associated with a 2.5 mL dosage is 27 lbs. Labeling should be revised accordingly, or the registrant should provide additional information (such as the amount of dosage actually dispensed by an applicator) justifying the proposed dosages and associated weight ranges.

   The only definite adverse effects were very slight (barely perceptible) erythema observed in all test substance groups and pin point bleeding present in a single dog in group 2 following the 30-day treatment.

   Although not stated in the report or investigators' conclusions, it is noteworthy that most of the adverse effects (barely perceptible erythema in a number of dogs, mostly in Group 4, and pin point bleeding for the first 3 time points on day 30 in one group 2 [1x] dog) occurred following the second (day 30) application of the test material. The only adverse effects following the first application (day 0) were in one group 3 dog which showed very slight (barely perceptible) erythema at 3 and 4 hours following application and at the AM observation on day 1. From the information provided in the report, it is not immediately

apparent as to why these minor adverse effects were more common following the second treatment.

A group 2 male (DF5 B71) ate only 0-25% of the food offered on day 0, and a group 3 female (CBC 683) ate only 0-25% of the food offered on days 1, 2, 3 and 12 (CBC 683, along with two group 4 dogs, had been identified as "obese" in pre-clinical examinations). These were the only post application occurrences of 0-25% food consumption.

There were a number of purely cosmetic effects, including spiking ("wet paint brush effect"), white deposits on hair tips, and scaling, which cannot be considered as indicative of toxicity.

There were no indications of effects body weight or hematology and clinical chemistry parameters.

From the 870.7200 Guidelines: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..." The effects seen in this study (barely perceptible erythema, pinpoint bleeding in one dog after receiving a 1X application) were both transient and non-life-threatening. **On this basis, the study can be classified as acceptable in supporting the use of this product on adult (>6 months old) dogs.**

Refer to the attached DER for additional comments.

2. The 44-day companion animal safety study (MRIDs 49788721, 49866901) with beagle puppies (49-51 days old at the start of the study, weights ranging from 1.08 to 3.27 kg on day -1) has been classified as acceptable. This study supports the proposed use on puppies 7 weeks of age and older, although the proposed minimum weight should be raised to 5 lbs, and the minimum weight associated with a 2.5 mL dosage is 27 lbs. Labeling should be revised accordingly, or the registrant should provide additional information (such as the amount of dosage actually dispensed by an applicator) justifying the proposed dosages and associated weight ranges.

   No mortality occurred. All puppies survived to the end of the study.

   Individual daily observations are reported on pages 13-152 of MRID 49866902. Post-application findings are summarized on p. 30 of MRID 49788722. Findings (for both groups) included loose feces, eye discharge and diarrhea. One group 4 puppy had slight inappetance on day 1 and another had diarrhea and was listless on day 1. Both of these puppies recovered by day 2.

   From information on pages 40-42 of MRID 49788722 three group 1 puppies and nine group 4 puppies received medications for coccidia prophylaxis after day 0.

There were no indications of any effect(s) associated with exposure to the test material with respect to food consumption, body weights, or body weight gains. There were no indications of any dose-related effects involving hematology or clinical chemistry parameters.

There were cosmetic effects (spiking, greasiness, deposits on tips on hair), but no indications of pruritis and/or erythema.

The study author concluded [p. 8 of MRID 49788722] that: "The Test Substance T2, containing imidacloprid, permethrin and pyriproxyfen, administered twice within a 30-day interval at 5x the recommended dose was safe to use under the conditions of the study. An adequate margin of safety was indicated between the control group and the 5X dose as there were no toxic signs recorded in any of these groups."

This reviewer is in agreement with the stated conclusions of the study author with respect to the lack of toxicity that occurred in beagle puppies at 5x the recommended dose. In addition, the proposed minimum age of 7 weeks is supported by this study. However, the proposed label dosages and weight bands are not entirely supported by this study.

According to the proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

The proposed minimum weight on the label of 91384-G is 4 lbs. From information on p. 1376 the mean weight of the four lowest weight male and four lowest weight female Group 4 (5X) puppies on day -1 was 1.96 ± 0.46 kg (4.33 ± 1.01 lb), so the 5X dosage rate was 1.02 mL/kg (0.46 mL/lb). [From information on p. 1375 the mean weight of the four lowest weight male and four lowest weight female Group 1 puppies was 1.63 ± 0.38 kg (3.60 ± 0.84 lbs), so that lower weight puppies were available]. The mean 5X application rate of 1.02 mL/kg (0.46 mL/lb) supports a 1X application rate of 0.204 mL/kg or 0.0926 mL/lb. Rounding up from 4.33 lbs, it is concluded that the minimum weight supported by this study for a dosage of 0.4 mL is 5 lbs, and that the minimum weight associated with a 2.5 mL dosage is 27 lbs. The labeling should be revised accordingly, or the registrant should provide additional information (such as the amount of the product actually dispensed by an applicator) justifying the proposed dosages and associated weight ranges.

The study is classified as acceptable, provided the labeling is revised (or otherwise addressed) as indicated above.

Refer to the attached DER for additional comments.

EPA Reviewer: Byron T. Backus, Ph.D., Toxicologist
CITAB, Registration Division (7505P)

Signature: Byron T Backus
Date: July 27, 2016

EPA Secondary Reviewer: Masih Hashim, Ph.D.
CITAB, Registration Division (7505P)

Signature: _____
Date: _____

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study; adult dogs; OPPTS 870.7200

PC CODE[S]: 129099 (Imidacloprid: 8.67%); 109701 (Permethrin: 45.21%); 129032 (Pyriproxyfen: 0.42%)

DP BARCODE: 432710

TEST MATERIAL (PURITY): T2, Batch No. T2MD04; containing (from p. 19 of MRID 49788721) Imidacloprid: 8.67% w/w; Permethrin: 45.21% w/w; and Pyriproxyfen: 0.42% w/w. In an acute oral LD_{50} study (see p. 15 of MRID 49788715) with a different batch number (T2MD06, containing 44.97% Permethrin, 8.71% Imidacloprid, and 0.43% Pyriproxyfen) the test material is described as a liquid with a specific gravity of 1.144.

SYNONYM[S]: T2; T2.200 for Dogs

CITATION[S]: MRID 49788721: Erasmus, H. (2015) A Target Animal Safety Study of T2 Applied Topically to Adult Dogs Final Report. Project Number: CV/15/154, PN1767. Unpublished study prepared by ClinVet International (Pty) Ltd. 1963p.

MRID 49866901: Erasmus, H. (2016) A Target Animal Safety Study of T2 Applied Topically to Adult Dogs: Final Report. Project Number: CV/15/154, PN1767. Unpublished study prepared by ClinVet International (Pty) Limited. 253p.)

SPONSOR: (p. 11 of MRID 49788721): Omnipharm Limited, BioCity, Nottingham, UK

SUBMITTER: CAP IM SUPPLY, INC

EXECUTIVE SUMMARY: In a 44-day companion animal safety study (MRIDs 49788721, 49866901), T2 (Batch No. T2MD04), containing 8.67% w/w Imidacloprid; 45.21% w/w Permethrin; and 0.42% w/w Pyriproxyfen, was applied topically as a spot-on on Days 0 and 30 of the study. There were four groups (each consisting of 6 males and 6 females) of dogs. Group 1 (controls) received a 5X dose of mineral oil; Group 2 (1X) received a single dose of test material; Group 3 (3X) received a 3X dose of test material; and Group 4 (5X) received a 5X dose of test material. From p. 22 of MRID 49788721: "The Test/Control Substance were administered using hypodermic syringes without a needle. The correct dose volumes were drawn directly from the supplied Test/Control Substance container or were decanted into smaller

containers to prevent contamination of the supplied containers… The Test/Control Substance dose was applied topically, divided into two to four spots on the dorsal midline from the shoulders to the base of the tail… Dogs weighing up to 9.5 kg received two spots, dogs weighing >9.5 kg to 25 kg received three spots and dogs weighing more than 25 kg received four spots… The Test/Control Substance was applied directly to the skin through parting the hair until the skin was visible… Care was taken not to spill any product. No product was spilled… Dogs were restrained by hand for approximately one minute following Test/Control Substance administration, to prevent any possible run-off of the product. No run-off occurred."

From p. 21 of MRID 49788721: "Multiple doses were applied in divided doses over a period of no more than two hours." From p. 10 of MRID 49866901: "The use of multiple doses was never implemented. Due to the size of the dogs versus the Test Substance volumes applied, this was not needed. It is acknowledged that the wording in the Final Study Report does not accurately reflect this." From p. 8-10 of MRID 49788721 each Group 1 dog received 12.5 mL mineral oil on Days 0 and 30, with the exception of one female (EA0 FF6; 8.1 kg on Day -5), which received 5.0 mL mineral oil on Days 0 and 30. Each Group 2 dog received 2.5 mL test material on Days 0 and 30, each Group 3 dog received 7.5 mL test material on Days 0 and 30, and each Group 4 dog received 12.5 mL test material on Days 0 and 30.

No mortality occurred. All animals survived to the end of the study.

The study author states the following (p. 8 of MRID 49788721):

"The only Adverse Events (AEs) that could be regarded as related to the administration of the Test Substance were very slight erythema (barely perceptible) recorded in all Test Substance groups and pin point bleeding present in a single animal (5B3 E6F) in group 2. The erythema was dose related, since groups 2 and 3 had one affected animal each and group 4 had six affected animals. Group 1 [controls] had no affected animals. The pin point bleeding was an individual reaction as it occurred after administration in a single animal only. This dog also had slight erythema at the first pin point bleeding observation.

"The recommended dose for Test Substance T2, containing imidacloprid, permethrin and pyriproxyfen, administered twice within a 30 day interval at 1x, 3x and 5x, was safe to use under the conditions of the study."

From p. 11 of MRID 49866901 very slight (barely perceptible) erythema was observed in one Group 2 dog at 1 hour on Day 30, in one Group 3 dog at 3 and 4 hours on Day 0 and at the AM observation on Day 1, and in six Group 4 dogs at 1 hour on Day 30. One of the six Group 4 dogs also had very slight erythema at 2 and 3 hours on Day 30. One Group 4 dog (4E1 CA6) had erythema at two sites (midback and tailbase); the other Group 4 dogs had erythema at only one application site (either the tailbase or behind shoulder blades).

Individual daily observations are reported on p. 13-253 of MRID 49866901. The following events are listed (summarized on page 34 of MRID 49788721): Page 64: Group 1: Day 15: Dog

EA0 FF6: Vomiting; Page 99: Group 2: Day 15 (PM): Dog 5B8 FA7: Limping hind leg; Page 107: Group 2: Day 39 (AM): Dog 5BE 0DD: Vomiting; Page 164: Group 3: Day 13 (PM) to Day 15 (AM): Dog 5CD 48E: Slight limping (broken toenail); Page 243: Group 4: Day 39 (AM & PM): Dog CCF C02: Left hind foot limp.

On page 33 of MRID 49788721 it is stated that: "Other observations include pin point bleeding behind shoulder blades present in one animal (5B3 E6F) in group 2 on Day 30 for the first three time points." However, no signs ("NS") are reported for this dog on page 91 of MRID 49866901.

From p. 65 of MRID 49788721: "...pruritus (itching and scratching) was present in one animal in group 1 on Day 17. Pruritus was also present in one animal in group 3 on Day 18 at both timepoints."

Individual daily food consumption values are reported on pages 1805 to 1924 of MRID 49788721. From p. 1860 a Group 2 male (DF5 B71) consumed only 0-25% of the food offered on Day 0, and (from p. 1888) a Group 3 female (CBC 683) consumed 0-25% of the food offered on Days 1, 2, 3 and 12 (CBC 683, along with two Group 4 dogs, had been identified as "obese" in pre-clinical examinations). These were the only post application occurrences of 0-25% food consumption.

Individual body weights (taken on Days -5, -1, 9, 14, 29, 37) and body weight changes are reported on pages 1789 through 1796 of MRID 49788721. Most dogs (Group 1: 8/12; Group 2: 10/12; Group 3: 7/12; Group 4: 12/12) lost weight in the period from Day -1 to 9, with a maximum weight loss of 0.62 kg in a Group 3 female (CBC 683); weight losses in Group 4 dogs ranged from 0.02 to 0.40 kg (mean weight loss: 0.18 kg). It is concluded that there were no treatment-related effects on body weights or body weight changes.

There were no indications of any dose-related effects involving hematology or clinical chemistry parameters.

There were a number of purely cosmetic effects, including spiking ("wet paint brush effect"), white deposits on hair tips, and scaling, which cannot be considered as indicative of toxicity.

Although not stated in the report or investigators' conclusions, it is noteworthy that most of the adverse effects (barely perceptible erythema in a number of dogs, mostly in Group 4, and pinpoint bleeding for the first 3 time points on day 30 in one Group 2 dog) occurred following the second (day 30) application of the test material. The only adverse effects following the first application (day 0) were in one Group 3 dog (5C9 268), which showed very slight erythema (barely perceptible) at 3 and 4 hours following application and at the AM observation on day 1. From the information provided in this report, it is not immediately apparent as to why adverse effects were more common following the second treatment.

From the 870.7200 Guidelines: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..." The effects seen in this study (barely perceptible erythema, pinpoint bleeding in one dog after receiving a 1X application) were both transient and non-life-threatening. **On this basis, the study can be classified as acceptable in supporting the use of this product on adult (>6 months old) dogs.**

However, according to the proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

The proposed minimum weight on the label of 91384-G is 4 lbs. The 4 lowest weight group 4 males (13.9, 14.9, 15.7 & 16.8 kg) and the 4 lowest weight group 4 females (10.3, 10.8, 11.3 & 12.1 kg) had a mean weight of 13.23 kg, and were treated with a 5X dose of 12.5 mL test substance, or a dosage of 0.945 mL test substance/kg. This supports a maximum 1X dose of 0.189 mL/kg, or 0.086 mL/lb. Since 0.4 mL ÷ 0.086 mL/lb = 4.65 lb the minimum weight supported by this study for a dose of 0.4 mL is 5 lb (the puppy study in MRID 49788722 supports a slightly higher 1X dosage rate of 0.204 mL/kg or 0.0926 mL/lb, but because 0.4 mL ÷ 0.0926 mL/lb = 4.32 lb it would still have to be rounded up to 5 lb). The minimum weight associated with a 2.5 mL dosage (based on the puppy study in MRID 49788722) is 27 lbs.

This companion animal safety study in adult dogs (beagles) is **Acceptable** with the dosage rate revisions indicated above. It **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in adult dogs.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and [No] Data Confidentiality statements were provided for the original study report (MRID 49468209) as well as amendment two (MRID 49724101).

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: T2; T2.200 for Dogs

Description:	In an acute oral LD_{50} study (see p. 15 of MRID 49788715) with a different batch number (T2MD06, containing 44.97% Permethrin, 8.71% Imidacloprid, and 0.43% Pyriproxyfen) the test material is described as a liquid with a specific gravity of 1.144.
Batch #:	Batch No. T2MD04
Purity:	Imidacloprid: 8.67% w/w; Permethrin 45.21% w/w; and Pyriproxyfen: 0.42% w/w.
Compound Stability:	Date of Manufacture (from p. 20 of MRID 49788721): 12 May 2015. The test material, in brown glass bottles, was stored at room temperature, in the dark. Stored below 25°C and protected from the sunlight.
CAS #:	52645-53-1 (Permethrin); 138261-41-3 (Imidacloprid); 95737-68-1 (Pyriproxyfen)

2. **Control:** Mineral oil

Description:	Supplied by Sigma Aldrich
Batch #:	MKBQ1755V
Purity:	Not Provided
Compound Stability:	From p. 20 of MRID 49788721: Re-test date: September 2018. To be stored at 25°C.

3. **Test animals:**

Species:	Dog	
Breed:	Beagle	
Age/weight at study initiation:	10 months to 8 years and 3 months old on Day 0. [Note: individual ages are not provided]. Males: 13.4-23.5 kg; Females: 8.1-18.2 kg [From information on p. 32-33 of MRID 49788721 one group 1 dog (963 BA4), one group 3 dog (CBC 683) and two group 4 dogs (954 CB2 and CC3 6D1) were obese].	
Source:	From the ClinVet animal colony; individually identified by subcutaneous transponders, each with unique identification.	
Housing:	Individual in cages with a floor space of ~3.0 m x 2.1 m	
Diet:	From p. 18 of MRID 49788721: "Food was supplied once a day… Animals were fed an age appropriate commercial dog diet. Dogs under the age of 12 months were fed Eukanuba puppy medium breed (Reg. no. V15464) and dogs 12 months and older were fed VetsBrands Premium adult maintenance dog food (…VetsBrands Reg. No. V24369). One dog received Purina Husky Adult…as a supplement to its normal diet for four days, as prescribed by the attending Veterinarian, to stimulate its appetite after a clinical examination revealed slight abdominal sensitivity…"	
Water:	From p. 18 of MRID 49788721 potable water was replenished at least twice a day in stainless steel bowls.	
Environmental conditions:	**Temperature:**	From p. 18 of MRID 49788721 temperature was set at ~20°C ± 4. Deviations of more than ±2°C occurred on days 10 and 11.
	Humidity:	Not reported
	Air changes:	Not reported.
	Photoperiod:	From p. 17 of MRID 49788721: 12 hours light/12 hours dark
Acclimation period:	From p. 17 of MRID 49788721: "The animals were acclimatized…for a period of 14 days before the first administration of the Test/Control Substances."	

B. STUDY DESIGN:

1. **In life dates:** From p. 23-24 of MRID 49788721: Start: 12 May 2015: start of 14-day acclimation period; 26 May 2015: first administration of test/control substance; 25 June 2015: second administration of test/control substance; 9 July 2015 (day 44 of study): end of animal phase of the study.

2. **Animal assignment:** The study design is given in Table 1. From p. 18 of MRID 49788721 the study followed a randomized block design. The 48 dogs were ranked by sex in descending order of individual body weight, and were subsequently blocked into 12 blocks of four dogs each. Animal ID numbers (in ascending order) were used to break ties. From each block of four dogs, one dog was randomly allocated to each of the four groups. Allocation of animals to groups and administration of the test or control substance was the responsibility of non-blinded personnel. All other people involved in the study were blinded to the group allocation.

TABLE 1: Study design [a]

Test Group	Total Dosing Volume/Dog (Days 0 and 30)	Mean Dose (mg/dog)[c]		Dose (mg/kg)[d]		Number assigned	
		Permethrin	Imidacloprid	Permethrin	Imidacloprid	Males	Females
1. Control	12.5 mL[b]	0	0	0	0	6	6
2. 1X	2.5 mL	1293	248	91.27	17.51	6	6
3. 3X	7.5 mL	3879	744	271.9	52.15	6	6
4. 5X	12.5 mL	6465	1240	444.5	85.26	6	6

[a] Data derived from p. 31-32 of MRID 49788721.

[b] One dog (EA0 FF6; weight 8.1 kg) in the control group received 5.0 mL of the control substance on days 0 and 30.

[c] Calculated by reviewer, using a test substance specific gravity of 1.144 g/mL, and 45.21% (w/w) Permethrin and 8.67% (w/w) Imidacloprid (the 0.42% w/w Pyriproxyfen is not included in the calculations).

[d] Based on mean Day -1 weights (p. 63 of MRID 49788721) of 14.403 kg for Group 1, 14.166 kg for Group 2, 14.267 kg for Group 3, and 14.543 kg for Group 4.

The 4 lowest weight group 4 males (13.9, 14.9, 15.7 & 16.8 kg) and the 4 lowest weight group 4 females (10.3, 10.8, 11.3 & 12.1 kg) had a mean weight of 13.23 kg, and were treated with a 5X dose of 12.5 mL test substance, or a dosage of 0.945 mL test substance/kg. This supports a maximum 1X dose of 0.189 mL/kg, or 0.086 mL/lb. The puppy study in MRID 49788722 supports a slightly higher 1X dosage rate of 0.204 mL/kg or 0.0926 mL/lb.

3. **Dose selection rationale:** The doses in this study were consistent with the 1X dosages on p. 21 of MRID 49788721 (0.4 mL/dog weighing <5 kg (<11 lbs); 1.0 mL/dog weighing 5 kg to 9.5 kg (11 lbs to 21 lbs); 2.5 mL/dog weighing 9.5 kg to 25 kg (>21 lbs to 55 lbs); 4.0 mL/dog weighing >25 kg (>55 lbs). Since the dogs in Groups 2, 3 and 4 weighed from 9.9 kg to 21.9 kg they received either 2.5 mL (Group 2: 1X), 7.5 mL (Group 3: 3X) or 12.5 mL (Group 4: 5X). The doses (and associated weight ranges) given on the proposed label for 91384-G are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

4. **Treatment:** From p. 22 of MRID 49788721: "The Test/Control Substance were administered using hypodermic syringes without a needle. The correct dose volumes were drawn directly from the supplied Test/Control Substance container or were decanted into smaller containers to prevent contamination of the supplied containers… The Test/Control Substance dose was

applied topically, divided into two to four spots on the dorsal midline from the shoulders to the base of the tail… Dogs weighing up to 9.5 kg received two spots, dogs weighing >9.5 kg to 25 kg received three spots and dogs weighing more than 25 kg received four spots… The Test/Control Substance was applied directly to the skin through parting the hair until the skin was visible… Care was taken not to spill any product. No product was spilled… Dogs were restrained by hand for approximately one minute following Test/Control Substance administration, to prevent any possible run-off of the product. No run-off occurred."

From p. 21 of MRID 49788721: "Multiple doses were applied in divided doses over a period of no more than two hours." From p. 10 of MRID 49866901: "The use of multiple doses was never implemented. Due to the size of the dogs versus the Test Substance volumes applied, this was not needed. It is acknowledged that the wording in the Final Study Report does not accurately reflect this." From p. 8-10 of MRID 49788721 each Group 1 dog received 12.5 mL mineral oil on Days 0 and 30, with the exception of one female (EA0 FF6; 8.1 kg on Day -5), which received 5.0 mL mineral oil on Days 0 and 30. Each Group 2 dog received 2.5 mL test material on Days 0 and 30, each Group 3 dog received 7.5 mL test material on Days 0 and 30, and each Group 4 dog received 12.5 mL test material on Days 0 and 30.

5. **Statistics:** Food consumption: from p. 58 of MRID 49788721: "Daily food consumption was listed. Per group, the number of animals consuming their food in each of the categories was calculated over the following collection period: Day -13 to Day 44 and described using frequencies and percentages.

The categories were as follows:

Food consumption score (Fc):	Fc 1	0% to 25%;
	Fc 2	> 25% to 50%;
	Fc 3	> 50% to 75%;
	Fc 4	> 75% to 100%.

Body weight: from p. 58 of MRID 49788721: "The individual body weights and changes in body weights (absolute and percentage change) from baseline (Day -1) to the rest of the assessment days were calculated for each group and summarized using descriptive statistics. The groups were compared (2 vs 1, 3 vs 1 and 4 vs 1) with respect to the change from baseline in body weight on the post-administration days by an ANOVA with a group effect.

"An analysis of variance (ANOVA) of body weights was done to determine whether the groups differed significantly at baseline."

Specific pre- and post-administration observations: from p. 58 of MRID 49788721: "The local tolerance variables edema, erythema and eschar formation, hair effects, cosmetic changes, eye irritation and skin were listed per subject and tabulated using frequencies and percentages per group and time point."

Hematology and clinical chemistry: from p. 57 of MRID 49788721: "...the emphasis of the statistical analysis was on the change from baseline values in each of the hematology and clinical chemistry parameters. The magnitude of such changes were evaluated and presented descriptively..."

Reporting included the post-administration values that fell outside the reference range for specific laboratory parameters. In addition: "...post-administration values were compared to the baseline values in a within group comparison by means of an ANOVA with an animal and observation time (baseline, post-administration) as effects. Since the aim of the analysis was to statistically evaluate the significance of changes in parameters from baseline in conjunction with relevant clinical changes, a change from baseline that was statistically not significant ($p > 0.05$), did not necessarily indicate that the difference was not clinically relevant. Similarly, a statistically significant change from baseline should not have been necessarily interpreted as a clinically relevant finding, but should rather have been considered a finding that necessitated a careful review from a clinical point of view."

This reviewer considers the above-mentioned analyses to be acceptable.

C. METHODS:

1. Observations:

a. Post-dosing and daily observations: From p. 24 of MRID 49788721: "Specific post-administration observations were performed hourly ± 15 minutes for four hours after the end of each administration period (Days 0 and 30) and twice a day on Days 1 to 29 and Days 31 to 44... The observations included, but were not limited to, changes in skin, hair, eyes, mucous membranes, nervous signs and behavior patterns, as well as vomiting and diarrhea."

b. Clinical assessments: From p. 24 of MRID 49788721: "A veterinarian conducted a clinical examination on all dogs for enrollment and inclusion purposes... [from p. 23 of MRID 49788721 this clinical examination was on day -5]. These examinations included, but were not limited to, vital signs (pulse rate, respiratory rate and rectal temperature), mucous membranes, eyes, motility, lymph nodes, abdominal palpation, thoracic auscultation and skin condition.

There is no indication (p. 23-24 of MRID 49788721) that any clinical assessments were conducted following either the first (day 0) or second (day 30) application.

c. Application site observations: After treatment, the application site was observed twice daily for changes to the skin and fur. Any erythema/eschar and edema were scored according to the Draize scale, and the presence or absence of cosmetic changes to the hair, spiking (hair coming together in narrow, sharp points) and deposits (areas of test item visible on the surface), were also recorded.

2. **Body weight:** The dogs were weighed on days -5, -1, 0, 7, 14, 29, 37 and 44.

3. **Food consumption:** The amount of food offered daily to each dog, individual food consumption, as well as amount of food remaining, were recorded for days -14 through 44.

4. **Clinical pathology:** On days -14, 1, 7, 31 and 37 blood for hematology, clinical chemistry, and coagulation evaluation was collected. From p. 25 of MRID 49788721: "Blood specimens were collected om collection tubes for clinical chemistry on Days -14. 1 and 31. Blood specimens were also collected on Days 7 and 37 because abnormalities were recorded on Days 1 and 31. There is no indication that food was removed prior to collection.

The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count* (absolute and percentages)
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count		Reticulocyte count
	Blood clotting measurements		Morphology (if indicated)
X	(Activated Partial Thromboplastin time) (aPTT)*		Heinz body formation
	(Clotting time)		
X	\(Prothrombin time) (PT)*		

* Recommended for companion animals safety evaluation based on the 870.7200

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen (BUN)*
X	Phosphorus*		Cholesterol
X	Potassium*	X	Globulins*
X	Sodium*	X	Glucose (random)*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK or ALP)*	X	Direct bilirubin*
	Cholinesterase (ChE)**		Indirect bilirubin
	Creatinine phosphokinase	X	Total protein (TP)*
	Lactic acid dehydrogenase (LDH)		Triglycerides
X	Alanine aminotransferase (ALT/also SGPT)*		Serum protein electrophoresis
X	Aspartate aminotransferase (AST/also SGOT)*		Albumin/globulin ratio
	Sorbitol dehydrogenase		
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for a companion animal safety evaluation based on OPPTS 870.7200.
** Only recommended if one or more active ingredients in the formulation is a known cholinesterase inhibitor.

Reference ranges are provided (in conjunction with values not within reference ranges) for clinical chemistry (pages 40-49 of MRID 49788721) and hematology (pages 50-53 of MRID 49788721).

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

II. RESULTS

A. **ACTUAL DOSES ADMINISTERED:** The mg/kg doses of the active ingredients are given in Table 1.

B. OBSERVATIONS:

1. **Clinical signs:** Selected clinical signs data are given below (from Table H, p. 34 of MRID 49788721):

Group 1		
Day	**Animal ID**	**Observation**
15	EAO FF6	Vomiting
Group 2		
7	588 FA?	Vomiting
15		Limping hind leg
39	5BE ODD	Vomiting
Group 3		
13 to 15	5CD 48E	Slight limping (broken toenail)
Group 4		
39	CCF C02	Left hind foot limp

Group 1: Dogs received the control substance
Group 2: Dogs received the test substance (single dose), T2
Group 3: Dogs received the test substance (three times the dose), T2
Group 4: Dogs received the test substance (five times the dose), T2

The following occurrences of 0-25% consumption of the total food offered on a single day are reported (summarized from data on pages 1805 to 1925 of MRID 49788721):

TABLE 2. Occurrences in Which Dogs Consumed Only 0-25% of Total Food Offered		
Group	Dog Number & Sex	Days on which there was 0-25% Consumption of Total Food Offered
2	DF5 B71 (M)	0
3	288 E14 (F)	-5
3	5D4 15D (F)	-10
3	CBC 683 (F)	2, 3, 4, 12

From information on p. 1794 of MRID 49788721 CBC 683 weighed 15.60 kg on Day -1 and 14.98 kg on Day 9, a loss of 0.62 kg. This was the maximum weight loss for any dog in any group during this time period.

With the possible exceptions of DF5 B71 (Group 2) and CBC 683 (Group 3) there was no indication of a test-related effect on food consumption.

2. **Local effects at the application site:**

From p. 34, 66 and 67 of MRID 49788721 pruritus (itching and scratching) was present in one Group 1 dog on day 17, and in one Group 3 dog on Day 18 at both time points. Very slight erythema was seen in one dog (5B3 E6F) in Group 2 on Day 30 at 1 hour [this dog also had pin point bleeding behind the shoulder blades on Day 30 for the first three time points, see below]. In Group 3 one dog had very slight erythema (barely perceptible) on Day 0 at 3 and 4 hours post-administration and on Day 1 at the first observation. In Group 4, six dogs had very slight (barely perceptible) erythema on Day 30 at one hour post-administration, with one dog still showing very slight erythema at two and three hours post-administration. All observations were at the application sites.

One Group 2 (1X) dog (5B3 E6F) had pin point bleeding behind the shoulder blades on Day 30 for the first three time points. From p. 34 of MRID 49788721: "This observation was not present on any other day or in any other group."

Other effects were cosmetic and included greasiness, spiking (wet paint brush effect), deposit on tips of hair, slight scaling, and scales (>2 mm x 2 mm). These occurred in all groups (including Group 1, which was treated with mineral oil).

4. **Mortality:** There were no deaths or moribund sacrifices.

C. **BODY WEIGHT AND WEIGHT GAIN:** Body weight data are given in Tables 3 and 4. There were no indications of any treatment-related effects on body weights or body weight gain.

TABLE 3: Mean body weight data group/sex for adult beagles treated with control/test material[a]				
Parameter/ Study day or interval	Dosage			
	Group 1 (Control) Mean Body Weight (kg) ± S.D.	Group 2 (1X) Mean Body Weight (kg) ± S.D.	Group 3 (3X) Mean Body Weight (kg) ± S.D.	Group 4 (5X) Mean Body Weight (kg) ± S.D.
Males				
Day -5	17.07 ± 3.39	16.83 ± 2.83	17.28 ± 2.89	17.01 ± 2.97
Day -1	16.55 ± 3.26	16.26 ± 2.87	16.70 ± 2.80	16.66 ± 2.94
Day 9	16.40 ± 3.14	15.99 ± 2.69	16.53 ± 2.84	16.45 ± 2.86
Day 14	16.60 ± 3.19	16.18 ± 2.62	16.71 ± 2.94	16.67 ± 2.91
Day 29	16.22 ± 2.82	16.05 ± 2.67	16.43 ± 3.00	16.59 ± 2.75
Day 37	16.28 ± 2.86	16.11 ± 2.73	16.51 ± 2.88	16.61 ± 2.88
Females				
Day -5	12.45 ± 3.33	12.54 ± 2.42	11.96 ± 2.03	12.83 ± 3.00
Day -1	12.25 ± 3.45	12.08 ± 2.27	11.83 ± 2.03	12.43 ± 2.89
Day 9	12.22 ± 3.50	12.03 ± 2.17	11.73 ± 1.79	12.29 ± 2.94
Day 14	12.27 ± 3.50	12.14 ± 2.16	11.61 ± 1.68	12.42 ± 3.02
Day 29	12.14 ± 3.64	11.98 ± 2.04	11.75 ± 1.64	12.37 ± 3.17
Day 37	12.18 ± 3.44	12.30 ± 2.20	11.91 ± 1.61	12.52 ± 3.19

[a] Calculated from individual body weights on pages 1792 through 1803 of MRID 49788721. Values are Mean ± Standard Deviation, with n=6 for all groups/sex.

TABLE 4: Mean weight changes group/sex of adult beagles treated with control or test material[a]				
Parameter/ Study day or interval	Dosage			
	Group 1 (Control) Mean Body Weight Change (kg) ± S.D.	Group 2 (1X) Mean Body Weight Change (kg) ± S.D.	Group 3 (3X) Mean Body Weight Change (kg) ± S.D.	Group 4 (5X) Mean Body Weight Change (kg) ± S.D.
Males				
BW change (kg):				
Days -1 to 9	-0.155 ± 0.225	-0.265 ± 0.226	-0.173 ± 0.150	-0.207 ± 0.129
Days 9 to 14	0.200 ± 0.078	0.193 ± 0.207	0.182 ± 0.137	0.222 ± 0.110
Days 14 to 29	-0.375 ± 0.472	0.130 ± 0.228	-0.280 ± 0.178	-0.048 ± 0.225
Days 29 to 37	0.060 ± 0.194	0.057 ± 0.265	0.078 ± 0.146	0.018 ± 0.155
Days 37 to 44	0.082 ± 0.107	0.057 ± 0.239	0.092 ± 0.175	0.050 ± 0.080
Days -1 to 44	-0.188 ± 0.689	-0.088 ± 0.327	-0.102 ± 0.318	0.005 ± 0.183
Females				
BW change (kg):				
Days -1 to 9	-0.033 ± 0.111	-0.048 ± 0.158	-0.102 ± 0.266	-0.143 ± 0.097
Days 9 to 14	0.052 ± 0.053	0.108 ± 0.198	-0.123 ± 0.192	0.135 ± 0.172
Days 14 to 29	-0.137 ± 0.283	-0.162 ± 0.390	0.137 ± 0.165	-0.048 ± 0.416
Days 29 to 37	0.040 ± 0.263	0.322 ± 0.196	0.073 ± 0.178	0.148 ± 0.182
Days 37 to 44	0.122 ± 0.095	-0.098 ± 0.203	0.030 ± 0.236	0.033 ± 0.125
Days -1 to 44	0.043 ± 0.353	0.122 ± 0.565	0.102 ± 0.362	0.125 ± 0.623

[a] Calculated from individual body weights on pages 1792 through 1803 of MRID 49788721. Values are Mean ± Standard Deviation, with n=6 for all groups/sex.

The following is from p. 129 of MRID 49788721:

The following table displays the p-values regarding the change from baseline (Day -1) comparison between the groups.

		p-values				
Parameter	Comparison	Day 9	Day 14	Day 29	Day 37	Day 44
Weight (kg)	2 - 1	0.4122	0.7167	0.6580	0.2233	0.6319
	3 - 1	0.5688	0.1796	0.7873	0.5119	0.6967
	4 - 1	0.2901	0.7839	0.3184	0.2554	0.4609

Group 1: Dogs received the control substance
Group 2: Dogs received the test substance (single dose), T2
Group 3: Dogs received the test substance (three times the dose), T2
Group 4: Dogs received the test substance (five times the dose), T2

There is no indication of any significant difference, although the p-values above were calculated only for all dogs (males and females) in each group [they should also have calculated for separate sexes]. However, given the relatively small weight changes shown in Table 4, it is unlikely there would be any statistical significance.

D. FOOD CONSUMPTION:

The following table shows incidences of food consumption ranges on the days (0 and 30) of application of the test material as well as the two subsequent days.

TABLE 5. Incidences of Food Consumption on Days of Application and Subsequent Two Days[a]

	Amount of Offered Food Consumed			
	0-25%	25-50%	50%-75%	75-100%
Group 1				
Day 0*	0/12	0/12	0/12	12/12
Day 1	0/12	0/12	2/12	10/12
Day 2	0/12	0/12	0/12	12/12
Day 30*	0/12	0/12	1/12	11/12
Day 31	0/12	0/12	2/12	10/12
Day 32	0/12	0/12	2/12	10/12
Group 2				
Day 0*	0/12	0/12	2/12	10/12
Day 1	1/12	1/12	1/12	9/12
Day 2	0/12	0/12	1/12	11/12
Day 30*	0/12	0/12	0/12	12/12
Day 31	0/12	0/12	1/12	11/12
Day 32	0/12	0/12	1/12	11/12
Group 3				
Day 0*	0/12	1/12	0/12	11/12
Day 1	0/12	0/12	0/12	12/12
Day 2	0/12	0/12	0/12	12/12
Day 30*	0/12	0/12	0/12	12/12
Day 31	0/12	0/12	1/12	11/12
Day 32	0/12	0/12	0/12	12/12
Group 4				
Day 0*	0/12	0/12	0/12	12/12
Day 1	0/12	0/12	0/12	12/12
Day 2	0/12	0/12	0/12	12/12
Day 30*	0/12	0/12	0/12	12/12
Day 31	0/12	0/12	0/12	12/12
Day 32	0/12	0/12	0/12	12/12

[a] Data taken from pages 110 – 117 of MRID 49788721.

* Days of Application

It is noteworthy that all dogs in Group 4 consumed the maximum amount of food (75-100%) on the days of application as well as the two subsequent days.

E. BLOOD ANALYSES:

1. Hematology and coagulation parameters:

From p. 38 of MRID 49788721: "Hematology results are described in Appendix B, Section 2.1. The frequency of values that were not within the reference ranges were tabulated in Appendix A, Table N... None of the values reported out of range were of clinical relevance."

After examining Appendix A, Table N (pages 50-53 of MRID 49788721) this reviewer concludes there was nothing of any clinical significance and there was no indication of an effect involving exposure to the test material.

2. Clinical chemistry:

The following significant clinical chemistry values are reported on p. 37 of MRID 49788721:

Table J Summary of clinical chemistry abnormalities

Group	ID	Parameter (reference range u/L)	Study day	Value
3	CBC 683	ALP (26 to 146)	37	549
		ALT (21 to 60)		558
4	954 CB2	ALP (26 to 146)	-14	137
			1	212
			7	228
			31	253
			37	228
	E9E E30	ALP (26 to 146)	-14	120
			31	203
			37	177

Group 3: Dogs received the test substance (three times the dose), T2
Group 4: Dogs received the test substance (five times the dose), T2

From p. 37 of MRID 49788721:

All of these animals were clinically healthy and had no other abnormalities.

None of the other values reported out of range were of clinical relevance.

The elevated ALP and ALT in CBC 683 (Group 3) were recorded once in the study.

In Group 4 ALP only was elevated in 954 CB2 on 5 occasions and in E9E E30 on three occasions.

These values are non-specific and not indicative of a disease or serious tissue damage.

These enzymes occur in different tissues and when a single enzyme is increased it does not necessarily indicate a specific condition. In liver disease more than one liver enzyme, particularly ALP and GGT, is expected to be elevated and if the test item caused such a condition a dose relationship is expected which is not the case in this study. ALP has various isoenzymes that can be elevated in liver disease, endogenous or exogenous corticosteroid activity, bone or intestinal conditions.

ALT is usually elevated with AST in liver disease but can also be elevated in muscle necrosis, corticosteroid activity, various drugs and trauma.

The animals were clinically healthy, no dose relationship is evident, and no liver condition or other adverse condition can be diagnosed from these figures.

Refer Clinical Pathology, fourth edition, Latimer, Mahaffey and Prasse.

A. INVESTIGATORS' CONCLUSIONS:

The study author states the following (p. 8 of MRID 49788721):

"The only Adverse Events (AEs) that could be regarded as related to the administration of the Test Substance were very slight erythema (barely perceptible) recorded in all Test Substance groups and pin point bleeding present in a single animal (5B3 E6F) in group 2. The erythema was dose related, since groups 2 and 3 had one affected animal each and group 4 had six affected animals. Group 1 [controls] had no affected animals. The pin point bleeding was an individual reaction as it occurred after administration in a single animal only. This dog also had slight erythema at the first pin point bleeding observation.

"The recommended dose for Test Substance T2, containing imidacloprid, permethrin and pyriproxyfen, administered twice within a 30 day interval at 1x, 3x and 5x, was safe to use under the conditions of the study."

B. REVIEWER'S COMMENTS:

Although not stated in the report or investigators' conclusions, it is noteworthy that most of the adverse effects (barely perceptible erythema in a number of dogs, mostly in Group 4, and pinpoint bleeding for the first 3 time points on day 30 in one Group 2 dog) occurred following the second (day 30) application of the test material. The only adverse effects following the first application (day 0) were in one Group 3 dog (5C9 268), which showed very slight erythema (barely perceptible) at 3 and 4 hours following application and at the AM observation on day 1. From the information provided in this report, it is not immediately apparent as to why adverse effects were more common following the second treatment.

From the 870.7200 Guidelines: "The targeted adequate margin of safety is 5X. Consideration will be given to products with less than a 5X margin of safety, depending on the severity of clinical signs of toxicity (e.g. transient, non-life-threatening signs)..." The effects seen in this study (barely perceptible erythema, pinpoint bleeding in one dog after receiving a 1X application) were both transient and non-life-threatening. **On this basis, we can classify the study as acceptable and as supporting the use of this product on adult (>6 months old) dogs.**

However, according to the proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

The proposed minimum weight on the label of 91384-G is 4 lbs. The 4 lowest weight group 4 males (13.9, 14.9, 15.7 & 16.8 kg) and the 4 lowest weight group 4 females (10.3, 10.8, 11.3 & 12.1 kg) had a mean weight of 13.23 kg, and were treated with a 5X dose of 12.5 mL test substance, or a dosage of 0.945 mL test substance/kg. This supports a maximum 1X dose of 0.189 mL/kg, or 0.086 mL/lb. Since 0.4 mL ÷ 0.086 mL/lb = 4.65 lb the minimum weight supported by this study for a dose of 0.4 mL is 5 lb (the puppy study in MRID 49788722 supports a slightly higher 1X dosage rate of 0.204 mL/kg or 0.0926 mL/lb, but because 0.4 mL ÷ 0.0926 mL/lb = 4.32 lb it would still have to be rounded up to 5 lb).

C. STUDY DEFICIENCIES:

While the study did not use a concurrent vehicle control group, this is not a requirement (only a recommendation) in the current 870.7200 Companion Animal Safety Guidelines.

There is no reporting of individual ages (the only information as to ages is on page 17 of MRID 49788721 which states ages ranged from 10 months to 8 years and 3 months on Day 0). In addition, from information on pages 32-33 of MRID 49788721 one group 1 dog (963 BA4), one group 3 dog (CBC 683) and two group 4 dogs (954 CB2 and CC3 6D1) were

obese. However, reporting of the ages of individual adult animals and exclusion of obese animals are not requirements that are specified in the 870.7200 Guidelines.

The following is the Acute Toxicity Data Evaluation Record (DER) for the companion animal (adult beagle) safety study submitted for EPA File Symbol 91384-G which was conducted on T2, Batch No. T2MD04

1. DP BARCODE: 432710				
2. PC CODES (of proposed product): 129099 (Imidacloprid: 8.67%); 109701 (Permethrin: 45.21%); 129032 (Pyriproxyfen: 0.42%)				
3. CURRENT DATE: July 27, 2016				
4. TEST MATERIAL: T2, Batch No. T2MD04; containing (from p. 19 of MRID 49788721) Imidacloprid: 8.67% w/w; Permethrin: 45.21% w/w; and Pyriproxyfen: 0.42% w/w. In an acute oral LD_{50} study (see p. 15 of MRID 49788715) with a different batch no (T2MD06, containing 44.97% Permethrin, 8.71% Imidacloprid, and 0.43% Pyriproxyfen) the test material is described as a liquid with a specific gravity of 1.144.				
Study/Species/Lab Study # /Date	**MRID**	**Results**	**Tox Cat**	**Core Grade**
Companion animal safety / dog (adult beagle) / Clin Vet International, Bloemfontein, South Africa / Project No. CV/15/154, PN1767/ December 3, 2015 / OCSPP 870.7200 Companion animal safety / dog adult beagle (Report Supplement) / Clin Vet International, Bloemfontein, South Africa / Project No. CV/15/154, PN1767/ March 18, 2016/ OCSPP 870.7200	49788721 49866901	There were 4 groups, each consisting of 6M & 6F adult (10 months to 8 years and 3 months on Day 0) beagles. Day -1 weights: M: 13.4-23.5 kg; F: 8.1-18.2 kg. Dogs were topically exposed to control or test substance on Days 0 & 30. Study went to day 44. Group 1 received 5x dose of mineral oil (12.5 mL, except 1 female which received 5.0 mL); Group 2 received 1x (2.5 mL) test substance; Group 3 received 3x (7.5 mL) test substance; Group 4 received 5x (12.5 mL). No mortality; all dogs survived to end of study. There were no effects on body weight, or hematology and clinical chemistry parameters. One Group 2 male ate only 0-25% of the food offered on Day 0, and a Group 3 female ate only 0-25% of the food offered on Days 1, 2, 3 and 12. These were the only post application occurrences of 0-25% food consumption. Adverse effects, almost all following 30-day treatment, were barely perceptible erythema in a number of dogs, mostly from Group 4, and pinpoint bleeding in one Group 2 dog, considered to be both transient and non-life threatening. Maximum 1X dose supported is 0.086 mL/lb, so minimum weight supported by 0.4 mL dose is 5 lb (4.65 lb rounded up).	N/A	A (with label revision)

n.d. = not determined; Core Grade Key: A =Acceptable, S = Supplementary, W = Waived, U = Unacceptable, D = Data Gap

EPA Reviewer: Byron T. Backus, Ph.D., Toxicologist
CITAB, Registration Division (7505P)

Signature: Byron T Backus
Date: July 27, 2016

EPA Secondary Reviewer: Masih Hashim, Ph.D.
CITAB, Registration Division (7505P)

Signature: SCR for
Date: July 27, 2016

Template version 02/06

DATA EVALUATION RECORD

STUDY TYPE: Companion Animal Safety Study; puppy; OPPTS 870.7200

PC CODE[S]: 129099 (Imidacloprid: 8.67%); 109701 (Permethrin: 45.21%); 129032 (Pyriproxyfen: 0.42%)

DP BARCODE: 432710

TEST MATERIAL (PURITY): T2, Batch No. T2MD06; containing (from p. 21 of MRID 49788722) Imidacloprid: 8.71% w/w; Permethrin: 44.97% w/w; and Pyriproxyfen: 0.43% w/w. In an acute oral LD_{50} study (see p. 15 of MRID 49788715) with the same batch number (T2MD06) the test material is described as a liquid with a specific gravity of 1.144.

SYNONYM[S]: T2; T2.200 for Dogs

CITATION[S]: MRID 49788722: Erasmus, H. (2015) A Target Animal Safety Study of T2 Applied Topically to Puppies Final Report. Project Number: CV/15/155, PN1767. Unpublished study prepared by ClinVet International (Pty) Ltd. 1478p

MRID 49866902: Erasmus, H. (2016) A Target Animal Safety Study of T2 Applied Topically to Puppies: Final Report. Project Number: PN1767, CV/15/155. Unpublished study prepared by ClinVet International (Pty) Limited. 152p.

SPONSOR: (from information on pages 3 and 12 of MRID 49788722): Omnipharm Limited, BioCity, Pennyfoot Street, Nottingham, UK

SUBMITTER: CAP IM SUPPLY, INC

EXECUTIVE SUMMARY: In a 44-day companion animal safety study (MRIDs 49788721, 49866901), T2 (Batch No. T2MD04), containing 8.67% w/w Imidacloprid; 45.21% w/w Permethrin; and 0.42% w/w Pyriproxyfen, was applied topically on Days 0 and 30 as a spot-on. There were two groups (each consisting of 6 males and 6 females) of beagle puppies (49-51 days old at the start of the study, weights ranging from 1.08 to 3.27 kg on day -1).

Group 1 (controls) received a 5X dose of mineral oil and Group 4 (5X) received a 5X dose of test material. From p. 22-23 of MRID 49788722: "The Test/Control Substance was administered using hypodermic syringes without a needle. The correct dose volumes were drawn directly

from the supplied Test/Control Substance container… The Test/Control Substance dose was applied topically, divided in two to four spots on the dorsal midline from the shoulders to the base of the tail. All pups weighed less than 9.5 kg and received two spots. Multiple doses were applied in divided doses over a period of no more than two hours to the pups in groups 1 and 4."

From p. 9 of MRID 49866902: "Only one animal (57B 202 in group 4) on Day 30 received its Test Substance in two doses of 3.0 mL and 2.0 mL, 12 minutes apart at the same site of administration… The reason for this was that this was one of the smallest *[actually it weighed 4.91 kg, but it was receiving a total dose of 5.0 mL rather than the 2.0 mL that most others received]* puppies…and it was considered that applying a split dose would be appropriate in order to avoid runoff, which could have resulted in an incomplete dose being administered. The site of administration was not allowed to dry before the second administration, since based on past experience, this normally takes longer than the allowed two hours."

No mortality occurred. All puppies survived to the end of the study.

Individual daily observations are reported on pages 13-152 of MRID 49866902. Post-application findings are summarized on p. 30 of MRID 49788722. Findings (for both groups) included loose feces, eye discharge and diarrhea. One group 4 puppy had slight inappetance on day 1 and another had diarrhea and was listless on day 1. Both of these puppies recovered by day 2.

From information on pages 40-42 of MRID 49788722 three group 1 puppies and nine group 4 puppies received medications for coccidia prophylaxis after day 0.

Individual daily food consumption values are reported on pages 1382 to 1441 of MRID 49788722. There were 7 post-treatment (day 0 to day 44) occurrences of 0-25% food consumption in group 1 puppies, and 8 occurrences in group 2 puppies, with no indications of any effect(s) associated with exposure to the test material.

Puppies were weighed on days -1, 7, 14, 29, 37 and 44. There were no indications of any treatment-related effects on body weights or body weight gains.

Incidences of "local" (application site?) effects are reported on pages 115-161 of MRID 49788722. Only cosmetic effects (spiking, greasiness, deposits on tips on hair) were observed. There were no observations of pruritis and/or erythema.

There were no indications of any dose-related effects involving hematology or clinical chemistry parameters.

The study author concluded [p. 8 of MRID 49788722] that: "The Test Substance T2, containing imidacloprid, permethrin and pyriproxyfen, administered twice within a 30-day interval at 5x the recommended dose was safe to use under the conditions of the study. An adequate margin of

safety was indicated between the control group and the 5X dose as there were no toxic signs recorded in any of these groups."

This reviewer is in agreement with the stated conclusions of the study author with respect to the lack of toxicity that occurred in beagle puppies at 5x the recommended dose. In addition, the proposed minimum age of 7 weeks is supported by this study. However, the proposed label dosages and weight bands are not entirely supported by this study.

According to the proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

The proposed minimum weight on the label of 91384-G is 4 lbs. From information on p. 1376 the mean weight of the four lowest weight male and four lowest weight female Group 4 (5X) puppies on day -1 was 1.96 ± 0.46 kg (4.33 ± 1.01 lb), so the 5X dosage rate was 1.02 mL/kg (0.46 mL/lb). [From information on p. 1375 the mean weight of the four lowest weight male and four lowest weight female Group 1 puppies was 1.63 ± 0.38 kg (3.60 ± 0.84 lbs), so that lower weight puppies were available]. The mean 5X application rate of 1.02 mL/kg (0.46 mL/lb) supports a 1X application rate of 0.204 mL/kg or 0.0926 mL/lb. Rounding up from 4.33 lbs, it is concluded that the minimum weight supported by this study for a dosage of 0.4 mL is 5 lbs, and that the minimum weight associated with a 2.5 mL dosage is 27 lbs. The labeling should be revised accordingly, or the registrant should provide additional information (such as the amount of the product actually dispensed by an applicator) justifying the proposed dosages and associated weight ranges.

The study is classified as acceptable, provided the labeling is revised (or otherwise addressed) as indicated above.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and No Data Confidentiality Claims statements were provided for the original study report (MRID 49788722) as well as the report supplement (MRID 49866902).

I. MATERIALS AND METHODS

A. MATERIALS:

1.	**Test material:**	T2; T2.200 for Dogs
	Description:	In an acute oral LD_{50} study (see p. 15 of MRID 49788715) with the same batch number (T2MD06, containing 44.97% Permethrin, 8.71% Imidacloprid, and 0.43% Pyriproxyfen) the test material is described as a liquid with a specific gravity of 1.144.
	Batch #:	Batch No. T2MD06
	Purity:	Imidacloprid: 8.71% w/w; Permethrin 44.97% w/w; and Pyriproxyfen: 0.43% w/w.
	Compound Stability:	
	CAS #:	138261-41-3 (Imidacloprid); 52645-53-1 (Permethrin); 95737-68-1 (Pyriproxyfen)

2. Vehicle control: From p. 21 of MRID 49788722; Mineral oil

Description: No description provided

Batch #: (From p. 21 of MRID 49788722): MKBQ1755V

Purity: Not Provided

Compound Stability: No indication in the report that the vehicle control was tested for stability, although the expiration date is reported (p. 21 of MRID 49788722) as September, 2018. The control substance was stored at below 25.0°C.

3. Test animals:

Species: Dog

Breed: Beagle

Age/weight at study initiation: (From p. 17 of MRID 49788722): 49-51 days old; 1.08-3.27 kg on day -1.

Source: From the ClinVet animal colony. They were identified with subcutaneous transponders with unique alpha numeric codes

Housing: (From p. 17 of MRID 49788722): "Puppies were housed individually in suitably partititioned, individual, adult dog cages… Limited direct contact between two pups housed in the same adult dog cage was possible through the metal grid partition separating them. Only puppies in the same group were housed together in a divided adult cage…"

The floor size for each puppy was 1.5 m x 2.1 m."

Diet: (From p. 18 of MRID 49788722): "Food was supplied twice a day, with puppies receiving half of their daily ration in the morning and half in the afternoon… Animals were fed an age appropriate commercial dog diet Eukanuba puppy medium breed (Reg. no. V15464). Individual animals received Hill's puppy medium (Reg. no. V11863). Appropriate wet food (Purina Husky Puppy, Reg. no. V10430) was added by the Veterinarian for individual animals." From information on pp. 1382-1441 of MRID 49788722 the puppies were originally offered 0.5 cup of food/day, but this was increased on days 33 or 36 to 0.75 cup/day for 7 puppies in Group 1 and 7 puppies in Group 4.

Water: (From p. 18 of MRID 497887220: "…potable water was replenished at least twice a day in stainless steel bowls."

Environmental conditions:

- **Temperature:** (From p. 18 of MRID 49788722): temperature was set at 20° ± 4°C. Deviations for short periods of time occurred. (From p. 43 of MRID 49788722): "Temperatures in the cage environment ranged from 13.4°C to 27.0°C…"
- **Humidity:** (From p. 43 of MRID 49788722): "…relative humidity ranged from 17.4% to 59.4%."
- **Air changes:** Not reported. (There is a heading on p. 18 of MRID 49788722 for "Thermo-regulation and ventilation." However, there is no information as to ventilation or air changes).
- **Photoperiod:** 12 hours light/12 hours dark

Acclimation period: (From p. 18 of MRID 49788722): "The animals were acclimitised…for a period of at least 14 days before the first administration of the Test/Control Substances."

B. STUDY DESIGN:

1. **In life dates:** From p. 12 of MRID 49788722: "The study was conducted in phases as 7 week-old puppies became available." The first phase had an experimental start date (Day 0 = day of first application) of June 24, 2015. The second application day (Day 30) was July 24, 2015 and the termination date (Day 44) was August 7, 2015. The last phase (Phase 5) had a start date (Day 0) of August 19, 2015, a second application on September 18, 2015 and a termination (Day 44) date of October 2, 2015.

2. **Animal assignment:** The study design is given in Table 1. From p. 19 of MRID 49788722: "The study followed a randomized block design. The study was conducted in phases as the animals reached the inclusion age. Two tables were prepared, one for female pups and one for male pups. Both tables were divided into six blocks of two pups each to accommodate the 24 pups… As soon as a pup had reached the correct age, it was entered in the first available space of the first incomplete block, according to its sex. If more than one pup of the same sex had reached the inclusion age on the same day, they were ranked in ascending order of ID and entered into the table in that order."

TABLE 1: Study design [a]

Test Group	Total Dosing volume	Mean Dose (mg/puppy)[b]		Dose (mg/kg)[c]		Number assigned	
		Permethrin	Imidacloprid	Permethrin	Imidacloprid	Males	Females
1. Control	2.0 mL/puppy < 5 kg* 5.0 mL/puppy 5-9 kg 12.5 mL/puppy 9-25 kg	0	0	0	0	6	6
4. 5X	2.0 mL/puppy < 5 kg* 5.0 mL/puppy 5-9 kg 12.5 mL/puppy 9-25 kg	1027	199			6	6

* Since the puppies weighed 1.08-3.27 kg on Day -1, they were all dosed with 5 x 0.4 mL test/control substance on Day 0.

a Data derived from p. 22 of MRID 49788722.

c Calculated by reviewer, using a test substance specific gravity of 1.144 g/mL, and 44.9% (w/w) Permethrin and 8.71% w/w Imidacloprid.

From pages 8-9 of MRID 49866902 two controls, 698 0C0 (4.9 kg on Day 29) and 698 4D1 (6.05 kg on Day 29) each received 5.0 mL control item on Day 30, while four group 4 puppies, 5A3 1B0 (5.1 kg on Day 29), 5C3 CC8 (5.54 kg on Day 29), 5D1 0EA (4.94 kg on Day 29), and 57B 202 (4.91 kg on Day 29) each received 5.0 mL of the test substance.

3. **Dose selection rationale:** The 1X dosage level for a puppy < 5 kg in this study was 0.4 mL/kg. The proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb (1.81-4.54 kg) dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb (5.0-9.07 kg) dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb (9.53-24.9 kg) dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs (24.9 kg) and over. The study initially included two additional groups: a Group 2 (1X) and a Group 3 (3X). The puppies in these two groups presumably received a day 1 application of test material, but

were sham-treated with control material on day 30 (personnel conducting this study were blinded as to which dosage group individual puppies were in).

4. **Treatment:** From p. 23 of MRID 49788722: "The Test/Control Substance dose was applied topically, divided in two to four spots on the dorsal midline from the shoulders to the base of the tail. All pups weighed less than 9.5 kg and received two spots. Multiple doses were applied in divided doses over a period of no more than two hours to the pups in groups 1 and 4." From p. 9 of MRID 49866902: "Only one animal (57B 202 in group 4) received its Test Substance in two doses of 3.0 mL and 2.0 mL, 12 minutes apart at the same site of administration… The reason for this was that this was one of the smallest puppies in the phase, and it was considered that applying a split dose would be appropriate in order to avoid run off, which could have resulted in an incomplete dose being administered. The site of administration was not allowed to dry before the second administration, since based on past experience this normally takes longer than the allowed two hours."

   The following comment was previously made by this reviewer in a memorandum dated February 19, 2016 for 91384-G: "…draft labelling (submitted December 3, 2015) states (p. 11) that for dogs weighing 4-10 lbs and 11-20 lbs: "Apply the entire contents of the applicator to one spot as shown." This spot would be on the dog's back between the shoulder blades, so there is an inconsistency between the way the test/control materials were applied (to 2 spots) in this study and the directions for use. This inconsistency has to be addressed." The registrant has responded with the following (p. 10 of MRID 49866902): "As this was a safety study, the doses to be applied were 5x the standard dose that will be administered in practice. This obviously results in a much larger volume of product being applied than will happen in the field. Due to the small size of the puppies, the dose was administered in two spots rather than one, in order to minimise run-off and ensure that each puppy received its entire dose."

5. **Statistics:** From p. 53 of MRID 49788722: "The local tolerance variables of edema, erythema and eschar formation, hair effects, cosmetic changes, eye irritation and skin were listed per subject and tabulated using frequencies and percentages per group and time point."

   For body weight (p. 53 of MRID 49788722): "The individual body weights and changes in body weights (absolute and percentage change) from baseline (Day -1) to the rest of the assessment days were calculated for each group, and summarized using descriptive statistics. The groups were compared (4 vs 1) with respect to the change from baseline in body weight on the post-administration days by an ANOVA with a group effect."

   For food consumption (p. 53 of MRID 49788722): "Daily food consumption was listed. Per group, the number of animals consuming their food in each of the categories was calculated over the following collection period: Day -13 to Day 44 and described using frequencies and percentages."

   The categories were as follows:

Food consumption score (Fc):	Fc 1	0% to 25%
	Fc 2	> 25% to 50%
	Fc 3	> 50% to 75%
	Fc 4	> 75% to 100%

For clinical pathology (p. 52 of MRID 49788722): "...the emphasis of the statistical analysis was on the change from baseline values in each of the hematology and clinical chemistry parameters. The magnitude of such changes were evaluated and presented descriptively. The clinical relevance and interpretation from a clinical point of view were described in the study report..."

This reviewer considers these analyses to be acceptable.

C. **METHODS:**

1. **Observations:**

a. **General health observations:** From p. 25 of MRID 49788722 (daily observations): "These observations included, but were not limited to, habitus, color of urine, color and consistency of feces (dry, normal, soft, diarrhea, blood in feces), salivation, vomiting, skin lesions and an obvious change in general condition..."

b. **Clinical assessments:** From information on pages 23-24 of MRID 49788722 there were two pre-application clinical examinations on days -14 and -3 (±2). From p. 24: "These examinations included, but were not limited to, vital signs (pulse rate, respiratory rate and rectal temperatures), mucous membranes, eyes, motility, lymph nodes, abdominal palpation, thoracic auscultation and skin condition." There is no indication that there was any clinical examination following application of the control/test material.

c. **Application site observations:** After treatment, the application site was observed twice daily for changes to the skin and fur. Any erythema/eschar and edema were scored according to the Draize scale, and the presence or absence of cosmetic changes to the hair, spiking (hair coming together in narrow, sharp points) and deposits (areas of test item visible on the surface), were also recorded.

2. **Body weight:** The puppies were weighed on days -3 ± 2, -1, 7, 14, 29. 37 and 44.

3. **Food consumption:** The amounts of food offered daily to each dog and approximate percentages of offered food consumed were recorded for days -14 through +43.

4. **Clinical pathology:** On days -14 or -13, 1, 7, 31 and 37 blood for hematology, clinical chemistry, and coagulation evaluation was collected.

The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count* (absolute and percentages)
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
	Platelet count		Reticulocyte count
	Blood clotting measurements		Morphology (if indicated)
X	(Activated Partial Thromboplastin time) (aPTT)*		Heinz body formation
	(Clotting time)		
X	\(Prothrombin time) (PT)*		

* Recommended for companion animals safety evaluation based on the 870.7200 Guidelines.

b. Clinical chemistry:

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium		Urea nitrogen (BUN)*
X	Phosphorus*		Cholesterol
X	Potassium*		Globulins*
X	Sodium*	X	Glucose*
	ENZYMES	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Direct bilirubin*
	Cholinesterase (ChE)**	X	Indirect (or conjugated) bilirubin
	Creatinine phosphokinase	X	Total protein (TP)*
	Lactic acid dehydrogenase (LDH)		Triglycerides
X	Alanine aminotransferase (ALT/also SGPT)*		Serum protein electrophoresis
X	Aspartate aminotransferase (AST/also SGOT)*	X	Albumin/globulin ratio
	Sorbitol dehydrogenase	X	Urea
	Gamma glutamyl transferase (GGT)		
	Glutamate dehydrogenase		

* Recommended for a companion animal safety evaluation based on the 870.7200 Guidelines.
** Only recommended if one or more active ingredients in the formulation is a known cholinesterase inhibitor.

Reference ranges (associated with out-of-range values) are provided for hematology (pages 38, 80-91 of MRID 49788722) and clinical chemistry (pages 34-35 and 92-103 of MRID 49788722).

5. **Urinalysis:** Urinalysis is not required for companion animal safety studies and was not done as part of the current study.

6. **Sacrifice and pathology:** There were no deaths or moribund sacrifices during the study. Terminal sacrifices and gross necropsies were not done and are not required under OPPTS 870.7200.

II. RESULTS

B. OBSERVATIONS:

1. Clinical signs:

The following pre-exposure observations are reported on p. 31 of MRID 49788722:

Group	Day	Animal IDs	Observation
1	-7	698 OCO	Blood in faeces; faecal float negative
	-6 to -2		Lack of appetite
	-1		Improved
4	-11	578 202	Blood in faeces {watery)
	-10		Improved
	-7	5C3 CC8	Blood in faeces; faecal float negative
	-6		Blood in faeces
	-5		Improved
	-6 to -4	5A7 EOO	Loose faeces

The following post-exposure observations are reported on p. 30 of MRID 49788722:

Group 1			Group 4		
Day	Animal ID	All signs observed	Day	Animal ID	All signs observed
2	698 0C0	Coughing	1	5A2 CBA	Slight inappetance
4		Coughing	1	5A7 EOO	Listless; diarrhoea
5		Coughing	13	5A7 D8F	Loose faeces; listless
7	5A6 6BA	Loose faeces	14		Loose faeces
8		Loose faeces; bilateral eye discharge	19		Listless
9		Eye discharge – left	27, 28	5A7 EOO	Diarrhoea
14	698 27F	Loose faeces	37	5A4 2FD	Diarrhoea
15, 19		Diarrhoea			
26	5A6 AAC	Eye discharge			
27		Lacrimation			
40	698 27F	Diarrhoea			
39 - 44	5A6 6BA	Eye discharge			
Group 1: Dogs received the Control Substance Group 4: Dogs received the Test Substance (T2), at five times the recommended dose					

There is no indication in the post-exposure observations given above of any patterns consistent with toxicity from the test substance at 5X the recommended dose. From

information on pages 40-42 of MRID 49788722 three group 1 puppies and nine group 4 puppies received medications for coccidia prophylaxis after day 0.

2. **Food consumption:**

Individual daily food consumption values are reported on pages 1382 to 1441 of MRID 49788722. The initial daily ration was 0.5 cup/day/puppy. This was increased to 0.75 cup/day/puppy for 7 Group 1 and 7 Group 4 puppies on day 33 or 36.

Group 4 puppy 5A2 CBA is reported (p. 30 of MRID 49788722) as having slight inappetance on Day 1. From p. 1415 of MRID 49788722 this puppy consumed 0-25% of its ration on day 0 and 75-100% on day 1.

The following occurrences of individual daily food consumption values ranging from 0-25% are reported on pages 1382 to 1441 of MRID 49788722:

Group 1:

5A4 191 (M) Days -14, -13, -8, 5, 9
5A6 004 (F) Day -13
5A6 AAC (M) Days -13, -9, -4, 13
5B3 DA9 (M) Days -12, -11, -10, -8
697 FFA (F) Days -13, -12, 0, 1, 36
698 0C0 (F) Days -13, -12, -8, -7, -6, -2, -1, 1
698 27F (M) Days -14, -13, -12

Group 4:

5A2 CBA (F) Days -13, -8, -2, 0, 10
5A3 1B0 (M) Days -13, -12, -11
5A4 2FD (M) Days -14, -13, -12, -11, 11
5A5 8B9 (M) Day -9
5A7 D8F (M) Days -14, -12, 9, 18
5A7 E00 (F) Days -12, -11, -8, -6, -1, 0, 6
5A8 8F3 (F) Days -12, 40
5C3 CC8 (F) Day -11
5D1 0EA (F) Day -11

The puppies were evidently under stress (separation from the dam?) at the start of acclimation. Although two Group 4 puppies (5A2 CBA and 5A7 E00) had 0-25% food consumption on day 0 this was temporary (from p. 1415 of MRID 49788722 5A2 CBA had 75-100% food consumption on days 1 and 2, and from p. 1427 5A7 E00 had 25-50% consumption on day 2 and 50-75% on day 3).

From p. 58 of MRID 49788722: "Food consumption was inconsistent in both groups from Day -13 to Day -1. In group 1, more than 90% of the puppies consumed >75% to 100% of their prescribed amount of food (according to manufacturer's recommendations) on Day 0, compared with 75% in group 4. From Days 1 to 21, the percentage of puppies in group 1 who ate >75% to 100% of their food ranged from 41.7% to 91.7%, and was similar to group 4, which ranged from 50% to 100%. Both groups showed a steady improvement in food consumption during the 24-day period from Day 21 to Day 44. During that period in group 1, >75% to 100% food consumption was observed in 100% of the puppies on seven days, in >90% of the puppies on six days, in >80% of the puppies on 10 days and in >:70% of the puppies on one day. During that same [24-day] period in group 4, >75% to 100% food consumption was observed in 100% of the puppies on 12 days and in >90% of the puppies on 12 days... Based on...[these] observations, the groups did not differ with regard to food consumption from Day -13 to Day 20, and group 4 showed better food consumption from Day 21 to Day 44."

3. **Body weight and weight gain:**

The following means and standard deviations are calculated from individual body weight as reported on pages 1375 through 1380 of MRID 49788722.

Mean Body Weights (in kg) by Group and Sex						
	Day -1	Day 7	Day 14	Day 29	Day 37	Day 44
Group 1:						
Males	2.07 ± 0.69	2.45 ± 0.75	2.83 ± 0.90	3.96 ± 1.24	4.30 ± 1.24	4.79 ± 1.23
Females	1.82 ± 0.54	2.16 ± 0.71	2.59 ± 0.94	3.48 ± 1.25	4.01 ± 1.40	4.29 ± 1.44
Combined	1.94 ± 0.61	2.30 ± 0.72	2.71 ± 0.89	3.72 ± 1.22	4.16 ± 1.27	4.54 ± 1.31
Group 4:						
Males	2.25 ± 0.31	2.68 ± 0.31	3.07 ± 0.48	4.23 ± 0.69	4.77 ± 0.69	5.12 ± 0.68
Females	2.22 ± 0.77	2.57 ± 0.89	3.12 ± 0.97	4.08 ± 1.26	4.50 ± 1.18	4.99 ± 1.29
Combined	2.24 ± 0.56	2.62 ± 0.63	3.09 ± 0.73	4.15 ± 0.97	4.63 ± 0.93	5.06 ± 0.98

Mean Body Weight Gains (in kg) by Group and Sex					
	Day -1 to 7	Day 7 to 14	Day 14 to 29	Day 29 to 44	Day -1 to 44
Group 1:					
Males	0.38 ± 0.19	0.38 ± 0.20	1.12 ± 0.35	0.83 ± 0.10	2.72 ± 0.62
Females	0.34 ± 0.21	0.43 ± 0.23	0.90 ± 0.35	0.81 ± 0.27	2.47 ± 0.96
Combined	0.36 ± 0.18	0.41 ± 0.21	1.01 ± 0.35	0.82 ± 0.19	2.59 ± 0.78
Group 4:					
Males	0.43 ± 0.09	0.39 ± 0.24	1.16 ± 0.29	0.90 ± 0.22	2.88 ± 0.46
Females	0.35 ± 0.18	0.55 ± 0.15	0.96 ± 0.32	0.92 ± 0.20	2.77 ± 0.53
Combined	0.39 ± 0.14	0.47 ± 0.21	1.06 ± 0.31	0.91 ± 0.20	2.82 ± 0.48

From information on p. 1376 the mean weight of the four lowest weight male and four lowest weight female Group 4 puppies on day -1 was 1.96 ± 0.46 kg (4.32 ± 1.01 lb), so the study supports a minimum body weight of 5.00 lbs (rounding up from 4.32 lb).

Calculating individual doses (in mL/kg) for Group 4 puppies on Day 30 gives the following:

Females:

57B 202	1.0183
5A2 CBA	0.6452
5A7 B00	0.8811
5A8 8F3	0.5391
5C3 CC8	0.9025
5D1 0EA	1.0121

Males:

5A3 1B0	0.9804
5A4 2FD	0.5900
5A5 8B9	0.4474
5A7 D8F	0.5882
5BB 58F	0.4484
697 E35	0.4415

The mean of the 4 highest female values and 4 highest male values is 0.8026 mL/kg. Dividing this by 5 gives 0.1605 mL/kg, which is equivalent to 0.0729 mL/lb. The minimum weight associated with a 0.4 mL application supported by the 30-day data is then 0.4 mL ÷ 0.0729 mL/lb = 5.49 lb.

The minimum weight supported by the day 0 dosages is 5.00 lbs (rounded up from the 4.32 lbs obtained from the Day -1 bodyweights).

The following are the body weight statistics from p. 32 of MRID 49788722:

Group	Statistic	Baseline (Day -1)	Day 44	Change (Day 44)	%Change (Day 44)
1	n	12	12	12	12
	Mean	1.944	4.538	2.594	135.199
	SD	0.606	1.307	0.780	25.987
	Median	1.965	4.770	2.510	132.441
	Minimum	1.080	2.220	1.140	103.140
	Maximum	3.270	6.840	3.630	178.820
4	n	12	12	12	12
	Mean	2.235	5.058	2.823	131.011
	SD	0.561	0.982	0.480	26.550
	Median	2.235	5.320	2.750	125.018
	Minimum	1.100	3.300	2.200	103.170
	Maximum	3.140	6.790	3.650	200.000

Group 1: Negative control
Group 4: Dogs were treated topically with five times the dose of T2

The following are the p values associated with a comparison of bodyweights from Groups 1 and 4 (from p. 32 of MRID 49788722):

		p-values				
Parameter	Comparison	Day 7	Day 14	Day 29	Day 37	Day 44
Weight (kg)	4 - 1	0.6719	0.4497	0.5484	0.4650	0.3973

Group 1: Negative control
Group 4: Dogs were treated topically with five times the dose of T2

Overall there are no indications of any effect on bodyweights or bodyweight gains.

4. **Local effects at the application site:**

Incidences of "local" (application site?) effects are reported on pages 115-161 of MRID 49788722. Only cosmetic effects were observed, with the following incidences at 1 and 2 hours following application on Day 1 (from p. 115 of MRID 49788722):

Day	Time	Abnormality	Group 1	Group 4
0	1h	Spiking (wet paint brush effect)	12/12 (100.0%)	12/12 (100.0%)
	1h	Deposit on tips of hair	9/12 (75.00%)	12/12 (100.0%)
	1h	Greasy appearance	12/12 (100.0%)	9/12 (75.00%)
	1h	Slight scaling	0/12	0/12
	1h	Scales (>2mm x 2mm)	0/12	0/12
	1h	Pruritus (itching and scratching)	0/12	0/12
	1h	Very slight erythema (barely perceptible)	0/12	0/12
	1h	Other	0/12	0/12
0	2h	Spiking (wet paint brush effect)	12/12 (100.0%)	12/12 (100.0%)
	2h	Deposit on tips of hair	11/12 (91.67%)	12/12 (100.0%)
	2h	Greasy appearance	12/12 (100.0%)	9/12 (75.00%)
	2h	Slight scaling	0/12	0/12
	2h	Scales (>2mm x 2mm)	0/12	0/12
	2h	Pruritus (itching and scratching)	0/12	0/12
	2h	Very slight erythema (barely perceptible)	0/12	0/12
	2h	Other	0/12	0/12

Group 1: Control Substance group
Group 4: Dogs were treated topically with five times the dose of T2

The following incidences of cosmetic effects were observed on Day 1 (from p. 117 of MRID 49788722):

Day	Time	Abnormality	Group 1	Group 4
1	obs1	Spiking (wet paint brush effect)	8/12 (66.67%)	6/12 (50.00%)
	obs1	Deposit on tips of hair	6/12 (50.00%)	10/12 (83.33%)
	obs1	Greasy appearance	10/12 (83.33%)	2/12 (16.67%)
	obs1	Slight scaling	2/12 (16.67%)	6/12 (50.00%)
	obs1	Scales (>2mm x 2mm)	0/12	0/12
	obs1	Pruritus (itching and scratching)	0/12	0/12
	obs1	Very slight erythema (barely perceptible)	0/12	0/12
	obs1	Other	0/12	0/12
1	obs2	Spiking (wet paint brush effect)	7/12 (58.33%)	6/12 (50.00%)
	obs2	Deposit on tips of hair	6/12 (50.00%)	8/12 (66.67%)
	obs2	Greasy appearance	8/12 (66.67%)	2/12 (16.67%)
	obs2	Slight scaling	5/12 (41.67%)	6/12 (50.00%)
	obs2	Scales (>2mm x 2mm)	0/12	0/12
	obs2	Pruritus (itching and scratching)	0/12	0/12
	obs2	Very slight erythema (barely perceptible)	0/12	0/12
	obs2	Other	0/12	0/12

Group 1: Control Substance group
Group 4: Dogs were treated topically with five times the dose of T2

The following cosmetic effects were still present on Day 29 (from p. 145 of MRID 49788722):

Day	Time	Abnormality	Group 1	Group 4
29	obs1	Spiking (wet paint brush effect)	0/12	0/12
	obs1	Deposit on tips of hair	0/12	0/12
	obs1	Greasy appearance	0/12	0/12
	obs1	Slight scaling	3/12 (25.00%)	3/12 (25.00%)
	obs1	Scales (>2mm x 2mm)	0/12	1/12 (8.33%)
	obs1	Pruritus (itching and scratching)	0/12	0/12
	obs1	Very slight erythema (barely perceptible)	0/12	0/12
	obs1	Other	0/12	0/12
29	obs2	Spiking (wet paint brush effect)	0/12	0/12
	obs2	Deposit on tips of hair	0/12	0/12
	obs2	Greasy appearance	0/12	0/12
	obs2	Slight scaling	3/12 (25.00%)	3/12 (25.00%)
	obs2	Scales (>2mm x 2mm)	0/12	1/12 (8.33%)
	obs2	Pruritus (itching and scratching)	0/12	0/12
	obs2	Very slight erythema (barely perceptible)	0/12	0/12
	obs2	Other	0/12	0/12

Group 1: Control Substance group
Group 4: Dogs were treated topically with five times the dose of T2

All day 0 to day 44 incidences of pruritus (itching and scratching) and very slight erythema (barely perceptible) are reported as 0/12 for both Groups 1 and 2.

5. **Mortality:** There were no deaths or moribund sacrifices.

C. BLOOD ANALYSES:

1. Hematology and coagulation parameters:

The "most obvious" (p. 37 of MRID 49788722) individual out-of-range hematology parameter values are reported on p. 38 of MRID 49788722:

Parameter	Reference range	Animal ID	Day	Value recorded
Group 1				
White cell count (x10^9/L)	(10 - 23.6)	698 0C0	1	51.1
White cell count (x10^9/L)	(10 - 23.6)	698 0C0	7	48
Neutrophils Abs (x10^9/L)	(3.8 - 13.83)	698 0C0	1	28.16
Neutrophils Abs (x10^9/L)	(3.8 - 13.83)	698 0C0	7	33.94
Lymphocytes Abs (x10^9/L)	(4.21 - 7.95)	5B3 DA9	1	10.03
Lymphocytes Abs (x10^9/L)	(4.21 - 7.95)	698 0C0	1	17.63
Lymphocytes Abs (x10^9/L)	(4.21 - 7.95)	698 0C0	7	10.66
Lymphocytes Abs (x10^9/L)	(4.21 - 7.95)	698 27F	31	10.52
Monocytes Abs (x10^9/L)	(0.88 - 2.15)	698 27F	7	3.01
Eosinophils Abs (x10^9/L)	(0.1 - 0.57)	698 0C0	1	1.94
Basophils Abs (x10^9/L)	(0.02 - 0.11)	698 0C0	1	0.56
Basophils Abs (x10^9/L)	(0.02 - 0.11)	698 0C0	7	0.29
Platelet count (x10^9/L)	(249 - 847)	5A4 191	1	185
Platelet count (x10^9/L)	(249 - 847)	5A4 191	7	124
Platelet count (x10^9/L)	(249 - 847)	5A6 004	7	148
Prothrombin time (sec)	(5.8 - 46.7)	697 FFA	31	95.7
Patient aPTT (sec)	(10.1 - 17.2)	5A9 67F	31	23.1
Group 4				
Neutrophils Abs (x10^9/L)	(3.8 - 13.83)	5A7 E00	1	18.6
Prothrombin time (sec)	(5.8 - 46.7)	5A8 8F3	7	73.2
Prothrombin time (sec)	(5.8 - 46.7)	5A8 8F3	31	77.8
Prothrombin time (sec)	(5.8 - 46.7)	697 E35	7	66.1
Prothrombin time (sec)	(5.8 - 46.7)	697 E35	31	80.6

Group 1: Negative control
Group 4: Dogs were treated topically with five times the dose of T2

None of the above findings were considered to be clinically relevant.

Two Group 4 puppies had aPTT values that were below the reference range on day 1. On p. 37 of MRID 49788722 it is stated that the value for 5C3 CC8 could be a result of a gastrointestinal inflammation during the study, but that for 5D1 0EA was not accompanied by any clinical signs. From p. 57 of MRID 49788722:

Parameter	Animal ID	Group	Reference range	Day	Base value	End value	Change from baseline
Patient PTT (sec)	5C3 CC8	4	10.1 - 17.2	1	10.6	8.1	-2.5
	5D1 0EA	4	10.1 - 17.2	1	10.7	9.9	-0.8

Group 1: Control Substance group
Group 4: Dogs were treated topically with five times the dose of T2

It is stated (p. 37 of MRID 49788722) that: "The haematology results were not indicative of any test item related condition."

2. Clinical chemistry:

The "most obvious" (p. 33 of MRID 49788722) individual out-of-range clinical chemistry parameter values are reported on p. 34 (Group 1) and 35 (Group 4) of MRID 49788722. For Group 1:

Group	Parameter	Reference range	Animal ID	Day	Value recorded
1	Urea-S (mmol/L)	(2 - 4.9)	5A6 004	37	6.2
		(2 - 4.9)	5A6 6BA	1	6.3
		(2 - 4.9)	5A6 6BA	37	6.5
		(2 - 4.9)	697 FFA	31	6.5
		(2 - 4.9)	698 0C0	1	6.2
	Creatinine-S (umol/L)	(15 - 37)	5A3 923	1	55
		(15 - 37)	5A6 004	1	51
		(15 - 37)	5A6 6BA	1	59
		(15 - 37)	5A6 6BA	7	53
		(15 - 37)	5A6 004	37	51
		(15 - 37)	697 FFA	31	57
		(15 - 37)	698 27F	7	54
	Alk. phosphatase-S (u/L)	(108 - 198)	5A4 191	1	232
		(108 - 198)	5B2 F7C	1	240
		(108 - 198)	5B2 F7C	7	235
		(108 - 198)	5B2 F7C	31	256
		(108 - 198)	5B2 F7C	37	256
		(108 - 198)	698 27F	1	259
	ALT (SGPT) (u/L)	(10 - 33)	698 0C0	1	52
	AST (SGOT) (u/L)	(12 - 41)	5B2 F7C	1	55
		(12 - 41)	698 27F	37	66

Group 1: Negative control

For Group 4:

Group	Parameter	Reference range	Animal ID	Day	Value recorded
4	Urea-S (mmol/L)	(2 - 4.9)	57B 202	37	6.4
		(2 - 4.9)	5A2 CBA	1	6.3
		(2 - 4.9)	5A7 E00	1	6.1
		(2 - 4.9)	5A7 E00	31	7.5
	Creatinine-S (umol/L)	(15 - 37)	5A4 2FD	1	53
		(15 - 37)	5A7 E00	31	67
		(15 - 37)	5A7 E00	37	55
		(15 - 37)	5A8 8F3	1	53
	Bilirubin total-S (umol/L)	(2 - 3)	5A2 CBA	37	6
	Alk. phosphatase-S (u/L)	(108 - 198)	5A3 1B0	1	245
		(108 - 198)	5A3 1B0	7	228
		(108 - 198)	5A3 1B0	31	230
		(108 - 198)	5A3 1B0	37	236
		(108 - 198)	5D1 0EA	1	252

Group 4: Dogs were treated topically with five times the dose of T2

From p. 33 of MRID 49788722: "The clinical chemistry results were not indicative of any test item related condition."

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded [p. 8 of MRID 49788722] that: "The Test Substance T2, containing imidacloprid, permethrin and pyriproxyfen, administered twice within a 30-day interval at 5x the recommended dose was safe to use under the conditions of the study. An adequate margin of safety was indicated between the control group and the 5X dose as there were no toxic signs recorded in any of these groups."

B. **REVIEWER'S COMMENTS:** This reviewer is in agreement with the stated conclusions of the study author with respect to the lack of toxicity that occurred in beagle puppies at 5x the recommended dose. In addition, the proposed minimum age of 7 weeks is supported by this study. However, the proposed label dosages and weight bands are not entirely supported by this study.

According to the proposed label dosages are 0.014 fl. oz. (0.4 mL) for 4-10 lb dogs; 0.034 fl. oz. (1.0 mL) for 11-20 lb dogs; 0.084 fl. oz. (2.5 mL) for 21-55 lb dogs; and 0.135 fl. oz. (4.0 mL) for dogs 55 lbs and over. The maximum dosages associated with these four respective weight bands would then be 0.1 mL/lb, 0.091 mL/lb; 0.119 mL/lb, and 0.073 mL/lb.

The proposed minimum weight on the label of 91384-G is 4 lbs. From information on p. 1376 the mean weight of the four lowest weight male and four lowest weight female Group 4 (5X) puppies on day -1 was 1.96 ± 0.46 kg (4.33 ± 1.01 lb), so the 5X dosage rate was 1.02 mL/kg (0.46 mL/lb). [From information on p. 1375 the mean weight of the four lowest weight male and four lowest weight female Group 1 puppies was 1.63 ± 0.38 kg (3.60 ± 0.84 lbs), so that lower weight puppies were available]. The mean 5X application rate of 1.02 mL/kg (0.46 mL/lb) supports a 1X application rate of 0.204 mL/kg or 0.0926 mL/lb. Rounding up from 4.33 lbs, it is concluded that the minimum weight supported by this study for a dosage of 0.4 mL is 5 lbs, and that the minimum weight associated with a 2.5 mL dosage is 27 lbs. The labeling should be revised accordingly, or the registrant should provide additional information (such as the amount of dosage actually dispensed by an applicator) justifying the proposed dosages and associated weight ranges.

This companion animal safety study in puppies (beagles) is **Acceptable** with the dosage rate revisions indicated above. It **does satisfy** the guideline requirement for a companion animal safety study (OPPTS 870.7200) in 7-week old puppies.

C. **STUDY DEFICIENCIES:** As indicated above, because the mean weight of the four lowest weight male and four lowest female Group 4 (5X) puppies on day -1 was 1.96 kg (4.33 lb), the study does not support a minimum weight of 4 lbs. In order to support a 4 lb minimum weight claim, the mean weight of these eight puppies would have had to have been 1.81 kg.

The following is the Acute Toxicity Data Evaluation Record (DER) for 7-week beagle puppy safety study conducted on T2, Batch No. T2MD06 and submitted for EPA File Symbol 91384-G.

1. DP BARCODE: 432710				
2. PC CODES: 129099 (Imidacloprid: 8.71%); 109701 (Permethrin: 44.97%); 129032 (Pyriproxyfen: 0.43%)				
3. CURRENT DATE: July 27, 2016				
4. TEST MATERIAL: T2, Batch No. T2MD06; containing (p. 21 of MRID 49788722) Imidacloprid: 8.71% w/w; Permethrin: 44.97% w/w; and Pyriproxyfen: 0.43% w/w. In an acute oral LD_{50} study (p. 15 of MRID 49788715) with the same batch number (T2MD06) the test material is described as a liquid with a specific gravity of 1.144.				
Study/Species/Lab Study # /Date	**MRID**	**Results**	**Tox Cat**	**Core Grade**
Companion animal safety / dog (7-week old beagle puppies) / Clin Vet International, Bloemfontein, South Africa / Project No. CV/15/155, PN1767/ December 3, 2015 / OCSPP 870.7200 Companion animal safety / dog adult beagle (Report Supplement) / Clin Vet International, Bloemfontein, South Africa / Project No. CV/15/155, PN1767/ March 18, 2016/ OCSPP 870.7200	49788722 49866902	2 groups, each consisting of 6M & 6F beagle puppies (49-51 days old at the start of the study), 1.08-3.27 kg on day -1. Puppies were treated on Days 0 & 30. Group 1 (controls) were treated with a 5x dose of mineral oil and Group 4 (5X) received a 5X dose of test material. Study went to Day 44. Since all Group 4 puppies weighed 1.08-3.27 kg on day -1 they were all dosed with 5 x 0.4 mL test substance on Day 0. On Day 30 four group 4 puppies (4.91-5.54 kg on day 29) were dosed with 5 x 1.0 mL test substance; other 8 with 5 x 0.4 mL. Daily observations (both groups) showed occurrences of loose feces, eye discharge and diarrhea. One group 4 puppy had slight inappetance on day 1 and another had diarrhea and was listless on day 1. Both had recovered by day 2. There were no indications of any test material related effects on food consumption, body weights, or body weight gains. No indications of any dose-related effects on hematology or clinical chemistry parameters. Only cosmetic effects (spiking, greasiness, deposits on tips of hair) were observed, with no pruritus and/or erythema. Mean weight of 4 lowest weight males & 4 lowest females on day -1 was 1.96 kg so study supports dosage rate of 0.4 mL/1.96 kg = 0.4 mL/4.32 lbs. Rounding up would be 5 lbs, which is the minimum weight associated with 0.4 mL.	N/A	A with label revision

n.d. = not determined; Core Grade Key: A =Acceptable, S = Supplementary, W = Waived, U = Unacceptable, D = Data Gap